ATTACHMENT A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Yijun Ruan et al.
Serial No.:	10/664,234
Filed:	09/17/2003
For:	Method for Gene Identification Signature (GIS) Analysis
Group Art Unit:	1634
Examiner:	Johannsen, Diana B
Attorney Docket No.:	3240-0105

The undersigned, Bing Ren, in support of the above-identified patent application, hereby states as follows:

1. My name is Bing Ren, and I am a citizen of China and a permanent resident of the United States of America.

2. I provide a summary of my qualifications, career history and other achievements below and also a copy of my resume providing further details of these as Exhibit A.

3. I have a Ph.D. in Biochemistry and a M.S. in Computer Science in 1998, both from Harvard University. I also have a B.S. in Biophysics from the University of Science and Technology of China.

4. I am currently a Member of the Ludwig Institute for Cancer Research, San Diego Branch and a professor in the Department of Cellular and Molecular Medicine, University of California San Diego School of Medicine.

5. I have an extensive research background in the fields of molecular biology, genomics, expression analysis and bioinformatics.
6. I have published over 30 scientific articles and received a number of awards, in the course of my career to date, as detailed in Exhibit A.

7. I have successfully obtained research funding and been invited to give presentations at conferences, as detailed in Exhibit A. I also am listed as an inventor in US Patent 6410243.

8. I also teach at and serve on university committees at the University of California San Diego and hold several academic memberships and review grants for research funding bodies, as detailed in Exhibit A.

9. I have reviewed the specification and all of the claims pending in the above-identified patent application (i.e. claims 25-27, 29, 31-41, 44-50 and 53).

10. I have reviewed the prior art applied by the Examiner in the final Office Action issued on 2 March 2009, including US 6136537 (Maciewicz), Saha *et al.*, (Nature Biotechnology, 2002, 19:508-512), Belfort *et al* (Nucleic Acids Research, 1997, 25(17):3379-3388) and US 6054276 (Maciewicz II).

11. I believe that none of the above prior art discloses the subject matter of the claims currently pending in the above-identified patent application. I also believe that none of these references, whether considered individually or in combination, renders obvious the subject matter of the currently pending claims.

12. The claims currently pending in the above-identified patent application consist of four independent claims (claims 25, 26, 39 and 40) and nineteen dependent claims (27, 29, 31-38, 41, 44-50 and 54).

13. The currently pending independent claims require, among other elements, a full-length cDNA transcript for the method claimed.

14. More specifically, independent claim 25 currently reads as follows:

25. A method for preparing at least one ditag comprising:
(i) producing at least one full-length cDNA transcript, said transcript having a 5' terminus and a 3' terminus;
(ii) cleaving the full-length cDNA transcript at its 5' terminus to extract a 5' tag having a 5' end and 3' end and cleaving the full-length cDNA transcript at its 3' terminus to extract a 3' tag having a 5' end and 3' end; and
(iii) generating at least one ditag by ligating the 3' end of the 5' tag to the 5' end of the 3' tag; wherein the ditag comprises sequence information including the 5' start and 3' end of a full-length coding region of a gene.

15. Independent claim 26 currently reads as follows:

26. A method for preparing at least one ditag comprising:
(i) providing at least one full-length cDNA transcript having a 5' terminus and a 3' terminus; and flanked by two adapters;
(ii) cleaving the full-length cDNA transcript at its 5' terminus to extract a 5' tag having a 5' end flanked by an adapter and a 3' end and cleaving the full-length cDNA transcript at its 3' terminus to extract a 3' tag having a 5' end and a 3' end flanked by an adapter; and
(iii) generating at least one ditag flanked by two adapters by ligating the 3' end of the 5' tag to the 5' end of the 3' tag; wherein the ditag comprises sequence information including the 5' start and 3' end of a full-length coding region of a gene.

16. Independent claim 39 currently reads as follows:

39. A method for genome mapping, comprising:
preparing at least one ditag, the ditag including two joined first and second sequence tags, wherein the first tag includes the 5'-terminus sequence and the second tag includes the 3'-terminus sequence of a full-length coding sequence of a cDNA transcript, corresponding to the full-length coding region of a gene; mapping each of the two tags of the at least one ditag on the genome; and defining the structural region including exons and introns of the corresponding gene on the genome map.

17. Independent claim 40 currently reads as follows:

> 40. A method of gene discovery comprising: preparing at least one ditag, the ditag including two joined first and second sequence tags, wherein the first tag includes the 5'-terminus sequence and the second tag includes the 3'-terminus sequence of a full-length coding sequence of a cDNA transcript, corresponding to the full-length coding region of a gene; comparing the at least one ditag with a genome map and a gene database; detecting matching of the 5' and 3' termini tags on the genome map but detecting no match on one or more gene database.

18. The requirement for a full-length cDNA transcript is a significant feature of the invention. As understood by one of ordinary skill in the field of molecular biology, mRNA in eukaryotes is, in general, spliced to remove exons to produce a full-length mRNA transcript corresponding substantially to the full-length coding region of a gene. A polyA tail is also generally added to the mRNA post-transcriptionally. The polyA tail is not considered part of the coding region of the mRNA. In addition, the sequence of a cDNA transcript is generally provided as the sequence of the coding region. Accordingly, one of ordinary skill in the field would consider that a full-length cDNA transcript corresponds substantially to the full-length coding region of a full-length mRNA transcript. I understand that the method of the present invention relates to the preparation of ditags from full-length cDNA transcripts which include the starting and ending points of full-length coding sequences.

19. In the final Office Action issued on 2 March 2009, the Examiner relied on US 6136537 (Maciewicz), Saha *et al.*, (Nature Biotechnology, 2002, 19:508-512), Belfort *et al* (Nucleic Acids Research, 1997, 25(17):3379-3388) and US 6054276 (Maciewicz II) to reject the claims then pending in the application.

20. A thorough reading of Maciewicz does not reveal the preparation of ditags from full-length cDNA transcripts as defined in the present claims. The starting polynucleotide products (usually cDNAs) are digested by a frequent cutting restriction endonuclease

(Col 4, line 25-32). As a result of the fragmentation of the cDNA by this restriction, the starting and ending points of full-length cDNA transcripts are not ligated together in the ditags.

21. Further, Maciewicz at Col 6 states that "pairs of sequence tags may be obtained from cDNAs without cleavage by a restriction endonuclease; however, one of the sequence tags of each pair in such embodiments typically consists of a segment of the polyA tail of the cDNA and therefore lacks information content". Accordingly, Maciewicz acknowledges that one of the paired sequence tags in the ditag in this embodiment does not include nor represent the 3' ending point of the full-length coding sequence from a full-length cDNA transcript.

22. The Examiner indicated that Maciewicz explicitly teaches the use of full-length cDNAs at Col 8, line 56-Col 9, line 3. I note that this section discusses methods of preparing full-length cDNA but does not teach a method of preparing ditags including the 5' starting point and 3' endpoint of a full-length coding sequence from a full-length cDNA transcript. From reading Maciewicz, one of ordinary skill in the field would know to use a frequently cutting restriction enzyme on the full-length cDNA prior to preparing ditags from the restricted fragments of cDNA. Further, one of ordinary skill in the field would recognize that the 3' end of the full-length coding sequence of full-length cDNA transcript is not represented by ditags prepared from these cDNA fragments or ditags from full-length cDNA without initial restriction (as discussed above). It is evident that on a thorough reading, Maciewicz does not teach an enabling method of preparing paired ditags from full-length cDNA transcripts that include the 5' starting point and 3' ending point of the full-length coding sequence.

23. In addition, Maciewicz also suggests in Col 8, line 56-Col 9, line 3 of the specification the use of random priming as an alternative method of reducing 3' bias. However, with ditags prepared from randomly primed cDNA, the 3' ending point of the full-length coding sequence is unlikely to be included in and/or represented by one of the sequence tags in the paired ditag. In view of the above, Col 8, line 56-Col 9, line 3 of the specification does not disclose preparation of ditags comprising sequence information including the 5' starting point and 3' ending point of full-length coding sequence from full-length cDNA transcripts.

24. Further, with respect to claims 39 and 40 (and their respective dependent claims), Maciewicz also does not teach or suggest how to perform full-length cDNA transcript analysis or provide any data on this. The teaching of using frequently cutting restriction enzyme on the starting polynucleotide, the suggestion of using random priming does not teach, suggest or motivate one of ordinary skill in the field to arrive at a method of preparing paired ditags for characterizing the 5' starting point and 3' ending point of full-length cDNA transcript or the full-length of a gene.

25. Accordingly, the subject matter of pending claims 25-27, 29, 31-41 and 44-50 and 53 is not disclosed, taught or suggested by Maciewicz.

26. With respect to obviousness, Maciewicz does not teach the preparation of ditags from full-length cDNA transcripts as defined in the present claims, as discussed above. The above discussion with respect to Maciewicz is applicable to the obviousness rejections.

(i) Claim 32 was rejected for obviousness by the Examiner in view of Maciewicz and Saha *et al.* The Saha reference relates to single tags prepared by the SAGE method only and does not compensate for the deficiencies of Maciewicz. The Saha method does not disclose or suggest paired ditags of full-length cDNA transcripts nor the mapping of such paired ditags. Accordingly, the combination of Saha and Maciewicz does not suggest the method of pending claim 32 which relates to preparing ditag from full-length cDNA transcript and mapping the ditags.

(ii) Claim 36 was rejected for obviousness by the Examiner in view of Maciewicz and Belfort. However, as acknowledged by the Examiner, the Belfort reference is only relied upon only for homing restriction endonucleases. Belfort does not compensate for the deficiencies of Maciewicz. Accordingly, the combination of Belfort and other cited prior art (including Maciewicz) does not render the pending claim 36 obvious.

(iii) Claim 39 was rejected for obviousness by the Examiner in view of Maciewicz and Maciewicz II. Maciewicz II relates to a method of constructing a physical map by ordering pairs of sequences by matching identical sequences among the pairs. As acknowledged by the Examiner, Maciewicz II does not teach the use of cDNAs nor a

full-length cDNA transcript in the method and does not compensate for the deficiences of the primary reference Maciewicz. Accordingly, the combination of Maciewicz II and Maciewicz does not render pending claim 39 obvious.

(iv) The Examiner also alleged that claims 40-41 were obvious in view of Maciewicz in combination with Maciewicz II and Saha. The disclosures of Maciewicz II and Saha have also been discussed above and Saha, together with Maciewicz II do not compensate for the deficiencies of Maciewicz. Accordingly, claims 40-41 are not obvious to one of ordinary skill in the field in view of these documents.

27. Taking into consideration the extensive discussion already presented above on full-length cDNA transcript, none of the cited references Saha, Belfort and Maciewicz II taken alone or in combination with Maciewicz renders a method of preparing paired ditags comprising sequence information including the 5' end and 3' end of the full-length coding sequence from full-length cDNA transcript obvious.

28. The present inventors are able to prepare paired ditags that represent both transcript start and end sites on a transcriptome-wide scale. The method of the present invention prepares ditags from a full-length cDNA transcript corresponding substantially to the full-length coding region of a gene. The method of the present invention may thus identify transcription start and end sites of both known and uncharacterized genes, including alternative transcripts and aberrant transcripts from chromosomal rearrangements or other genetic changes in human cancers or other disorders. The method of the present invention lowers the costs of analyzing expressed genes from various cells and tissues. The present invention can be considered a significant contribution over the existing prior art and this invention is highly regarded by professionals in the field as discussed in Peters and Velculescu, Nature Methods, 2005, 2(2):93-94 (Exhibit B). I agree with the opinion of the authors of this paper on this method and add that this invention makes a significant contribution to gene expression analysis in its capability of analysing starting and ending points of transcription efficiently and for this reason, other investigators would be applying this technique widely in their research.

29. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these

statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

26 - August, 2009

Date

Bing Ren